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**APPROVAL PACKAGE FOR:**

**APPLICATION NUMBER  
21-064**

**Pharmacology Review(s)**

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

**KEY WORDS:** Ultrasound contrast agent, Perfluoropropane, Definity

**Reviewer Name:** Adebayo Laniyonu, Ph.D.

**Division Name:** Medical Imaging and Radiopharmaceutical Drug Products

**HFD#:** 160

**Review Completion Date:**

**Review number:** #3

**NDA number:** 21-064

**Submission:**

Serial Number	Letter Date	Stamp Date	Type of Submission
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	5/15/01	5/16/01	BP
000	5/30/01	5/31/01	BP

**Date:**

**Type of submission:** N

**Information to sponsor:** Yes ( ) No ( )

**Sponsor (or agent):**

DuPont Pharmaceuticals Company  
Medical Imaging Division  
331 Treble Cove Road  
North Billerica, MA 01862

**Manufacturer (drug substance):**

**Drug:**

**Code Names:** DMP 115, SG897, MRX-115 and Aerosomes  
**Generic Name:** Perfluoropropane  
**Trade Name:** Definity™  
**Chemical Name:** 1,1,1,2,2,3,3,3-Octafluoropropane  
**CAS Registry Number:** 76-19-7  
**Molecular Weight:** 188.02 g/mol

**Relevant INDs/NDAs/DMFs:**

IND #

DMFs #

**Drug Class:** Ultrasound Contrast agent

**Indication:** Echocardiography

**Clinical formulation:**

Vial Component	Concentration/mL
Sodium Chloride	6.8 mg
Propylene Glycol	103.5 mg
Glycerin	126.2 mg
Water for Injection, USP	
*Lipid Blend	0.75 mg
Sodium Hydroxide, NF	As needed to adjust pH
Hydrochloric Acid, NF	As needed to adjust pH
Perfluoropropane Gas	% in the Headspace
Injectate Characteristics	
Perfluoropropane Gas	0.15±0.10 mL
Number of Microbubbles (Clinically useful Range)	$1.2 \times 10^{10}$

**Route of administration:** Intravenous

**Previous clinical experience:** See medical officer review.

**Disclaimer use of sponsor's material:**

Sponsor submitted texts were utilized in the preparation of this review. They will be identified as quotes

**Studies reviewed with this submission:**

Study DRR-2001-22: Microvascular rheology of Definity™ microbubbles during arterial administration. Vol. 5. Pages 5-27

Study DRR 2001-01: A rising dose cardiovascular assessment of intravenously administered Definity in an acute model of pulmonary hypertension in the anesthetized closed-chest spontaneously breathing dog. Vol. 5. Pages 28-44

**Studies not reviewed:**

None

## **Introduction and drug history:**

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed for contrast-enhanced echocardiographic imaging of cardiac structure.

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator.

This submission (01/30/2001) by DuPont was in response to the agency's approvable letter of 8/4/2000. The agency advised the sponsor that the application was approvable for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border. The agency identified a number of issues to be addressed by the sponsor before the application is approved. The following non-clinical issues related to safety were identified. Please note that only the pre-clinical pharmacology aspect of the agency's letter is addressed. For a complete record of the agency's position on this product, please refer to the action letter NDA 21-064 of 04/08/2001.

## **FDA REQUEST:**

### **SAFETY**

1. In order to determine the risk of toxicity to patients with compromised pulmonary vasculature, as requested in the letter of October 8, 1999, a study in a chronically compromised pulmonary circulation disease model is still needed before approval. This should study a range of dose multiples based on body surface area. Also a range of times after activation should be studied.

Although we note the completion of the cremasteric muscle study to evaluate microvascular toxicity, this study used an intravenous injection. As such, the lungs filtered the larger particles before reaching the cremasteric muscle. Therefore, we request that this study be repeated using an intra-arterial injection.

## **SPONSOR'S RESPONSE:**

The sponsor agreed with the agency's position that Definity microbubbles may plug pulmonary microcirculation leading to right-sided failure and respiratory distress. To address this concern, the sponsor conducted two studies summarized below. The first study evaluated the effect of intra-arterially administered Definity on the microcirculation. The second study assessed the effects of intravenously administered Definity in an acute model of moderate and severe pulmonary hypertension. An evaluation of both studies will be provided following the studies summaries.

### **Study DRP-2001-22:**

**The sponsor** addressed the issue of the potential for DMP 115 to cause clumping, aggregation or coalescence in the systemic circulation in a study conducted by Jonathan, R. Lindner, MD and his co-workers at the University of Virginia school of medicine and titled:

#### **Microvascular rheology of Definity microbubbles during arterial administration:**

According to the sponsor, the specific aims of the study were to determine:

- 1) The extent of microvascular retention of Definity microbubbles following intrarterial injection
- 2) How deformability of Definity microbubble influences extent and duration of retention
- 3) Whether microbubble retention produces any detrimental effects on local hemodynamics or endothelial integrity.

Male Sprague-Dawley rat spinotrapezius muscle was prepared for intravital microscopic study. The muscle was exposed using a paramedian incision, and secured to a buffered pedestal. Fluorescence epi-illumination was performed using a 530-560 nm excitation filter. Centerline arteriolar blood cell velocities were measured using a custom designed program. Arteriolar flow was calculated as the product of mean velocity and cross sectional area. Definity microbubbles were labeled with a yellow fluorescent tag (PKH26) with a mean excitation wavelength of 551 nm.

Two protocols were utilized. In the first protocol, increasing doses of Definity 80- 800  $\mu\text{L/kg}$  ( $\times 0.6 - 6$  MHDbsa based on 20 $\mu\text{L/kg}$  dose) were injected via an arterial catheter at 15 minutes intervals. One minute following each injection, 20 optical fields encompassing several different arterioles were observed for static microbubbles. Microbubbles that were stationary for  $>1$  second were deemed stationary. For the second protocol, arterial pressure and blood flow within several arterioles were measured before, 30 sec and 5 minutes following intra-arterial injection of Definity 400 $\mu\text{L/kg}$  ( $\times 3$  MHDbsa). The numbers of static microbubbles were also measured 1, and 10 minutes following injection. Up to 4 injections were made in each animal.

The following results were reported:

- 1) The number of static microbubbles increased with increasing doses of Definity following arterial injection.

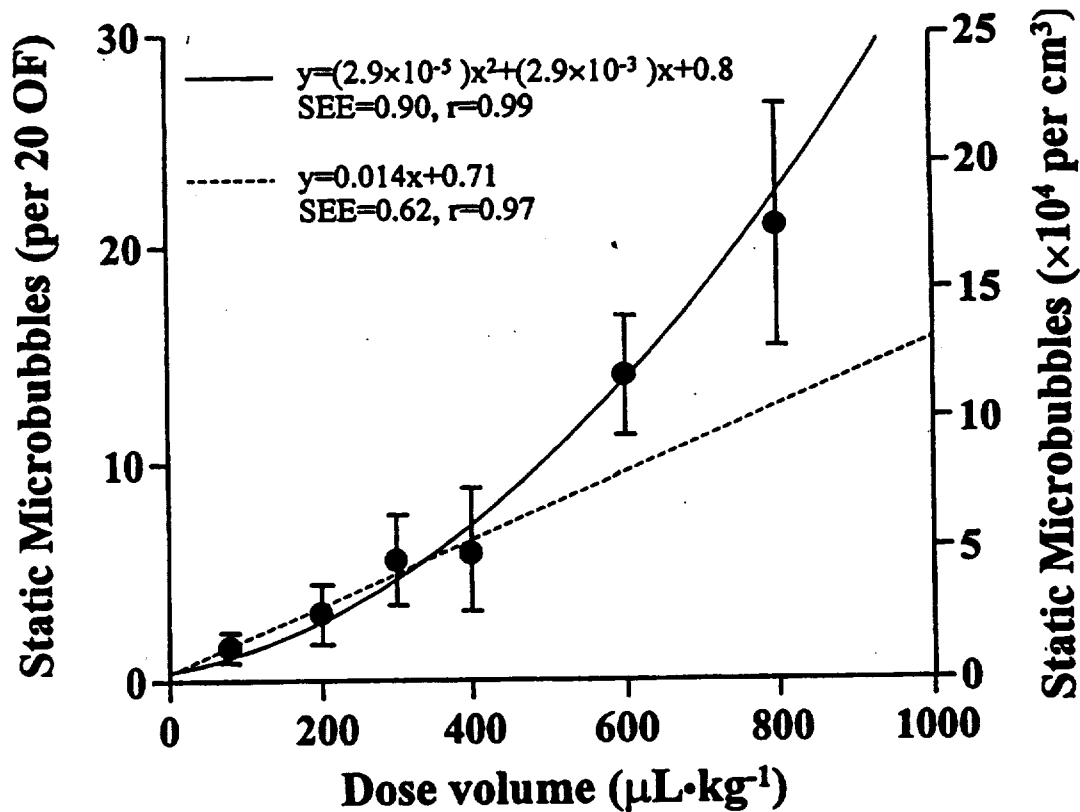


Fig.1: Relation between dose of Definity and the mean ( $\pm$ SD) number of static microbubbles detected in 20 optical fields (OF) by intravital microscopy of the spinotrapezius muscle (left y-axis) and per volume tissue (right y-axis) 1 minute after arterial injection. The solid line represents the quadratic fit to all data points whereas the dashed line represents the linear fit to the lowest 4 doses of Definity

- 2) Static microbubbles were seen in small arterioles  $<15\mu\text{m}$  especially at branch points, and in capillaries. RBC flux within the microvessel ceased transiently due to obstruction. Some RBC continued to pass round the microbubbles occasionally.
- 3) The mean retention fraction for Definity microbubble measured after 1 minute injection was about  $1.2 \pm 0.1\%$

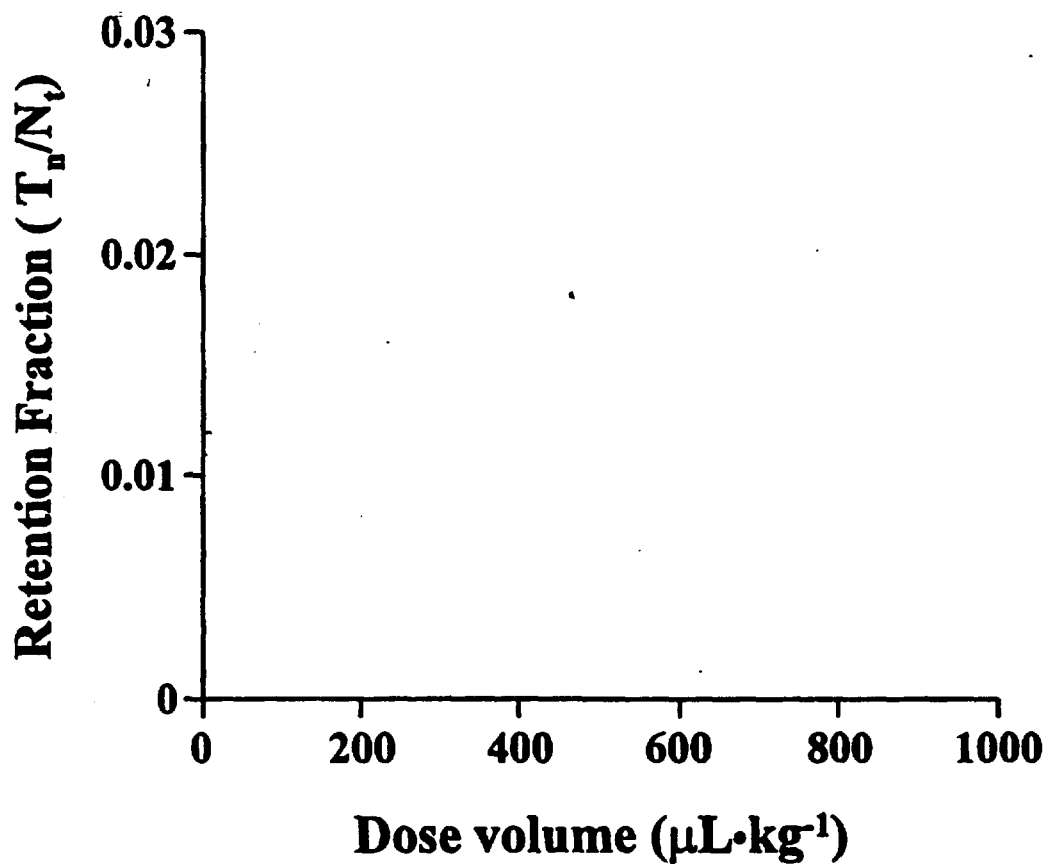


Fig.2: Retention fraction of Definity microbubbles in the spinotrapezius muscle, calculated from  $T_n/N_i$  ( $T_n$ = the number of microbubbles entrapped per  $\text{cm}^3$  of tissue in the spinotrapezius muscle for each dose of Definity 1 minute after arterial injection,  $N_i$  = the number of microbubbles entering  $1 \text{ cm}^3$  of tissue)

- 4) Majority of static microbubbles were  $>5\mu\text{m}$  in radial diameter with microbubbles of greater diameter being entrapped in larger vessels.

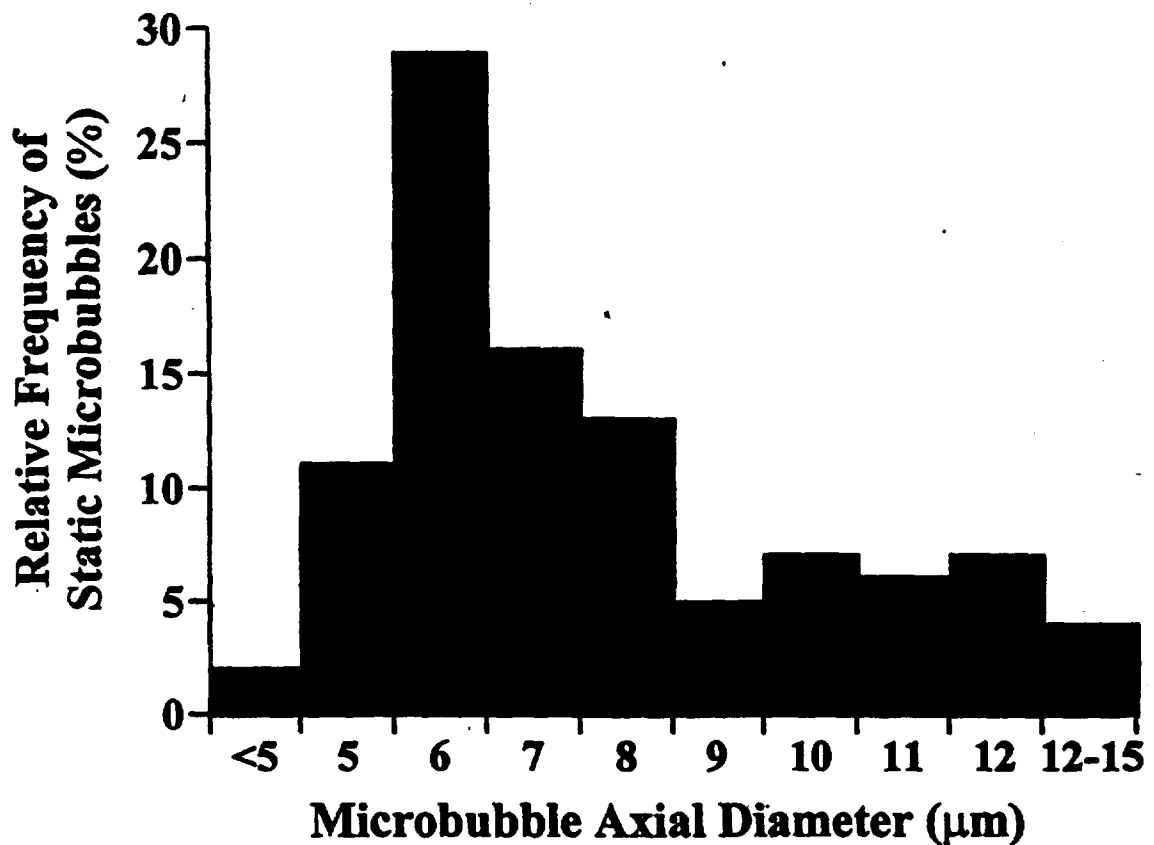


Fig.3: Histogram of the frequency of axial diameter for static microbubbles in the spinotrapezius muscle. The median axial diameter was 7μm.

- 5) Following entrapment, the vast majority of microbubbles became ellipsoidal.
- 6) Ability of the microbubbles to become deformed within the vessel was not dependent on microbubble size
- 7) There was no increase in microbubble size or coalescence over time.
- 8) Microbubble entrapment was transient in nature, decreasing significantly over a 10 minute period.



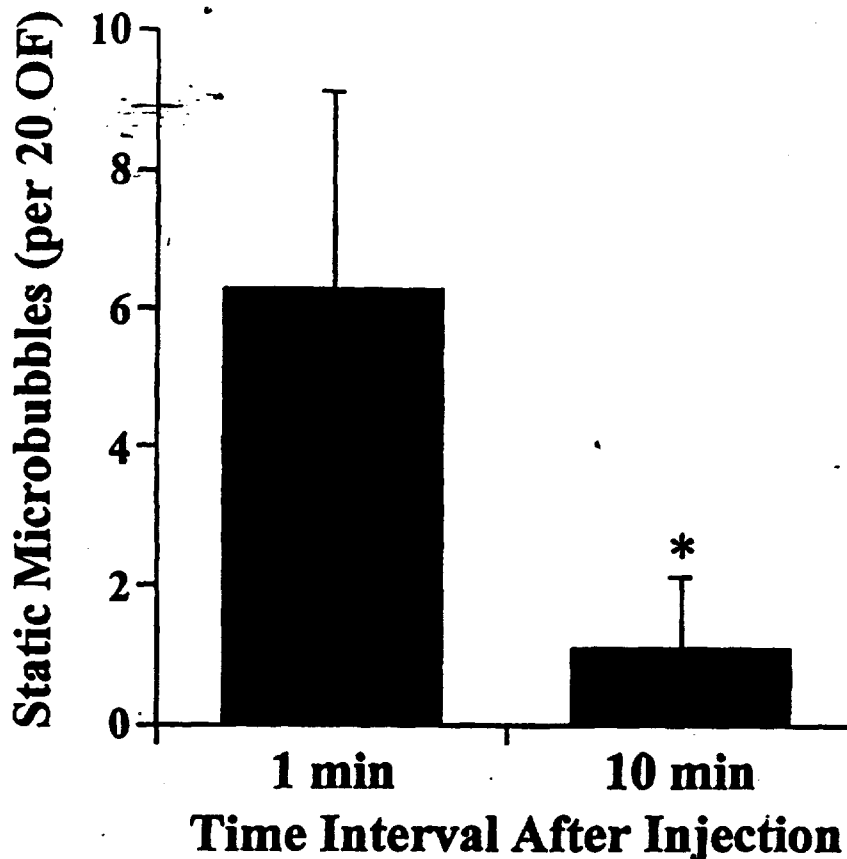


Fig.4: Mean (+SD) number of static microbubbles detected in 20 optical fields (OF) by intravital microscope of the spinotrapezius muscle 1 and 10 minutes after arterial injection \*P,0.05 compared with observations made 1 minute.

9) The mean arterial blood pressure and the mean blood flow within 18- 30  $\mu$ m arterioles selected a priori were not altered by microbubble injection.

**Table. Arterial Blood Pressure and Arteriolar Blood Flow Measurements at Baseline and Following Arterial Injection of 100  $\mu$ L Definity in the Rat-**

	Baseline	1 min	5 min
Mean arterial pressure (mm Hg)	95 $\pm$ 7	92 $\pm$ 9	92 $\pm$ 11
Arteriolar blood flow (nL $\cdot$ s <sup>-1</sup> )	164 $\pm$ 130	176 $\pm$ 136	152 $\pm$ 121

10) It was stated that there was no evidence of leukocyte or platelet adhesion at the site of entrapment

The sponsor concluded that a very small fraction (1.2%) of the injected microbubble were transiently retained within the microcirculation during their first pass. There were no detrimental effects of Definity microbubbles following intra-arterial injection. The sponsor also included an appendix (reproduced in full below) extrapolating the microcirculation results to humans.

From the appendix, the sponsor concluded that in humans, the fraction of lung capillaries transiently occluded immediately after injection of Definity 20  $\mu\text{l/kg}$  is 3.4%

**Reviewer's Comments:** The results of the present study demonstrated that a portion of Definity administered intra arterially become trapped transiently within the microcirculation. The outcome of this study is in contrast to that of an earlier study by DuPont conducted using intravenous route of administration. When Definity was administered intravenously, the results indicated that Definity microbubbles transit through normal capillaries as a single microbubble, and do not cause capillary or other microvascular obstruction.

The differing outcome between the two studies is a confirmation of the agency's position that studies evaluating microvascular toxicity should be conducted using an intrarterial mode of administration. Following intravenous administration, only microbubbles smaller than the functional diameter of pulmonary capillaries will enter the systemic circulation. The larger particles becoming filtered by the lungs.

It is noted that the microbubble entrapment is transient in nature, being reduced significantly at 10 minutes compared with the number of static microbubbles 1 minute following administration. The site of entrapment is in distal arterioles and capillaries, which perhaps will minimize effects on local oxygen delivery compared with entrapment occurring in larger arterioles since many distal capillary beds in a single region may become obstructed simultaneously.

The observation that most static microbubbles were  $> 5\mu\text{m}$  in radial diameter emphasizes the need for the agency to continue to encourage microbubble manufacturers on the need to reduce the total number of microbubbles with large diameters from their preparation.

Although the sponsor made a good faith attempt to extrapolate the results of the microcirculation study to the extent of microbubble retention in the human pulmonary microcirculation following venous administration of Definity. The sponsor concluded (see appendix B) that following Definity (20 µl/kg):

The total number of microbubbles transiently retained in the pulmonary circulation is  $3.7 \times 10^7$  or  $1.85 \times 10^7$  per lung.

The number of microbubbles transiently retained per  $\text{cm}^3$  of lung tissue was calculated to be  $2.4 \times 10^4 \text{ cm}^{-3}$

The fraction of capillaries transiently occluded immediately after injection is 3.4%.

I am hesitant to make such a direct extrapolation to humans, in view of the number of assumptions made in arriving at the numbers and lack of data to support them. The principal assumption, that the retention fraction observed in the rat spinotrapezius muscle (0.012) is the same as that in the human pulmonary vasculature is not supported by any data.

What is clear to this reviewer, is that there is microbubble entrapment in the microcirculation following intraarterial administration of Definity signifying that the lungs act as a filter allowing only the passage of microbubbles smaller than the diameter of pulmonary capillaries. The study has helped in risk identification especially in cases of patients with right-to-left, bidirectional, or transient right-to-left cardiac shunt where phospholipid-encapsulated microbubbles can bypass the pulmonary-filtering mechanisms and directly enter the arterial circulation. Definity should be contraindicated in such patients. The study has also opened up the theoretical consideration that the filtering mechanism by the lungs may lead to aggravation of pulmonary pathophysiology in patients with compromised pulmonary function.

**The study is deemed adequate.**

**Study DRR 2001-01:** A rising dose cardiovascular assessment of intravenously administered Definity in an acute model of pulmonary hypertension in the anesthetized closed-chest spontaneously breathing dog.

The stated goals of the study were to evaluate the effect of Definity on hemodynamics and myocardial contractility in a model of acute pulmonary hypertension in the anesthetized, spontaneously-breathing closed chest dogs.

For the study, dogs of both sexes (N= 4 per treatment group) were prepared for arterial, left ventricular, systemic arterial, and pulmonary arterial pressure, heart rate, myocardial contractility, respiration and EKG measurements. Arterial blood samples were taken to measure arterial pH,  $\text{PO}_2$  and  $\text{PCO}_2$ . Severe (sustained increase in mean PAP of 30 mm Hg above baseline) or moderate (sustained increase in mean PAP of 15 mm Hg above baseline) hypertension was induced with the injection of sephadex microsphere 11076 particles/ml (size range 100-600 µm) through the proximal lumen into the right atrium. Subsequently, Definity was administered at increasing doses of 40, 80, and 200 µl/Kg (X1, 2 and 5MHDbsa), IV at 30 minutes intervals.

The results of the study indicate that:

Acute pulmonary hypertension was induced by repeated injection of sephadex, however, sephadex injection did not alter systemic MAP or HR.

TABLE 1. Control (baseline) and post-treatment values of pulmonary arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in anesthetized dogs following injection of Sephadex G-50 suspension into the right ventricle.

	<b>Severe Pulmonary Hypertension</b>			
	<b>Vehicle Group (N=4)</b>		<b>Definity Group (N=4)</b>	
<b>Parameter</b>	<b>Control</b>	<b>Post-sephadex</b>	<b>Control</b>	<b>Post-sephadex</b>
<b>PAP (mm Hg)</b>	22 ± 3	49 ± 1*	18 ± 2	49 ± 3*
<b>ΔPAP from control</b>	—	27 ± 4	—	30 ± 2
<b>MAP (mm Hg)</b>	150 ± 3	132 ± 14	140 ± 7	149 ± 7
<b>HR (bpm)</b>	141 ± 6	140 ± 5	154 ± 4	153 ± 3
	<b>Moderate Pulmonary Hypertension</b>			
	<b>Vehicle Group (N=3)</b>		<b>Definity Group (N=4)</b>	
<b>Parameter</b>	<b>Control</b>	<b>Post-sephadex</b>	<b>Control</b>	<b>Post-sephadex</b>
<b>PAP (mm Hg)</b>	18 ± 5	33 ± 6*	17 ± 3	36 ± 4*
<b>ΔPAP from control</b>	—	15 ± 2	—	18 ± 2
<b>MAP (mm Hg)</b>	142 ± 9	142 ± 10	137 ± 10	146 ± 20
<b>HR (bpm)</b>	129 ± 15	141 ± 11	126 ± 12	136 ± 17

All values are mean ± SEM. Post-sephadex values were measured 30 min after administration of last dose of sephadex.

\*: Statistically significant ( $P < 0.05$ ; paired t-test) compared to corresponding control.

In the presence of severe or moderate PAP, Definity administration did not alter PAP, cardiac contractility, MAP, HR, corrected QT interval, respiratory rate, or arterial blood gases.

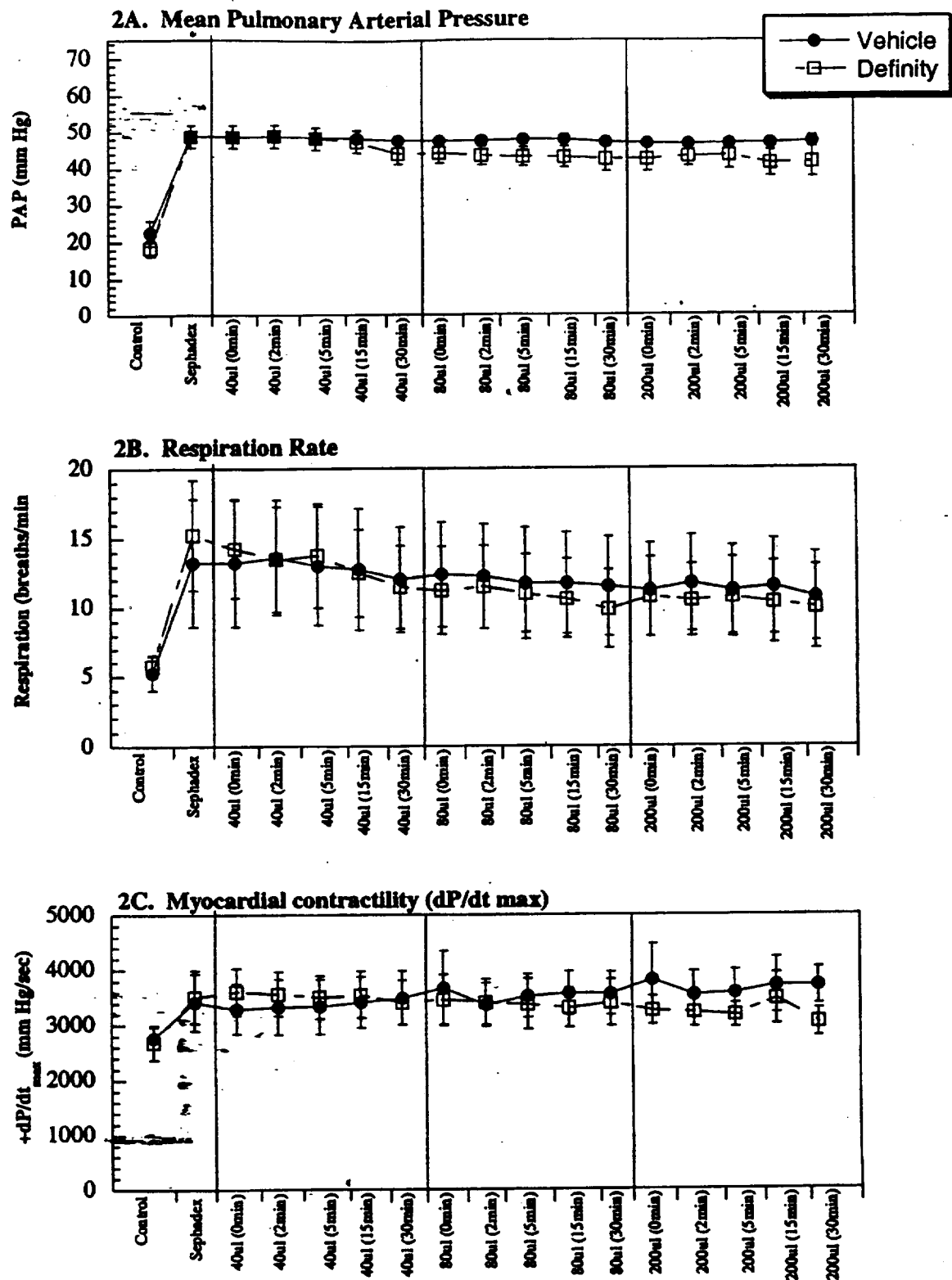


Fig.2: Effects of Definity and vehicle on (2A) mean pulmonary arterial pressure, (2B) respiration rate and (2C) myocardial contractility in spontaneous-breathing anesthetized dogs with severe pulmonary hypertension N=4/group.

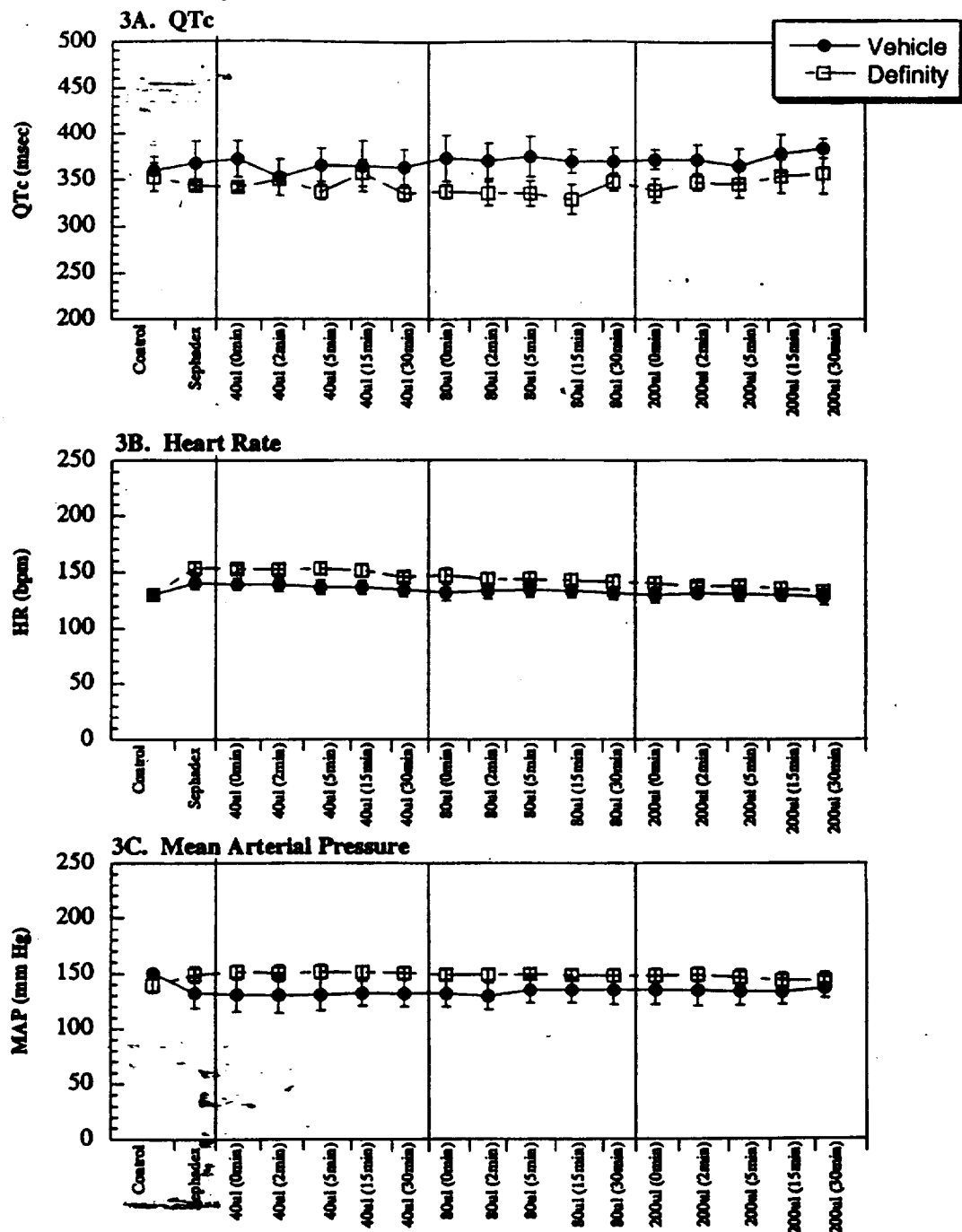


Fig.3. Effects of Definity and vehicle on (3A) QT<sub>c</sub>, (3B) heart rate and (3C) mean arterial pressure in spontaneously breathing anesthetized dogs with severe pulmonary hypertension (n=4/group)

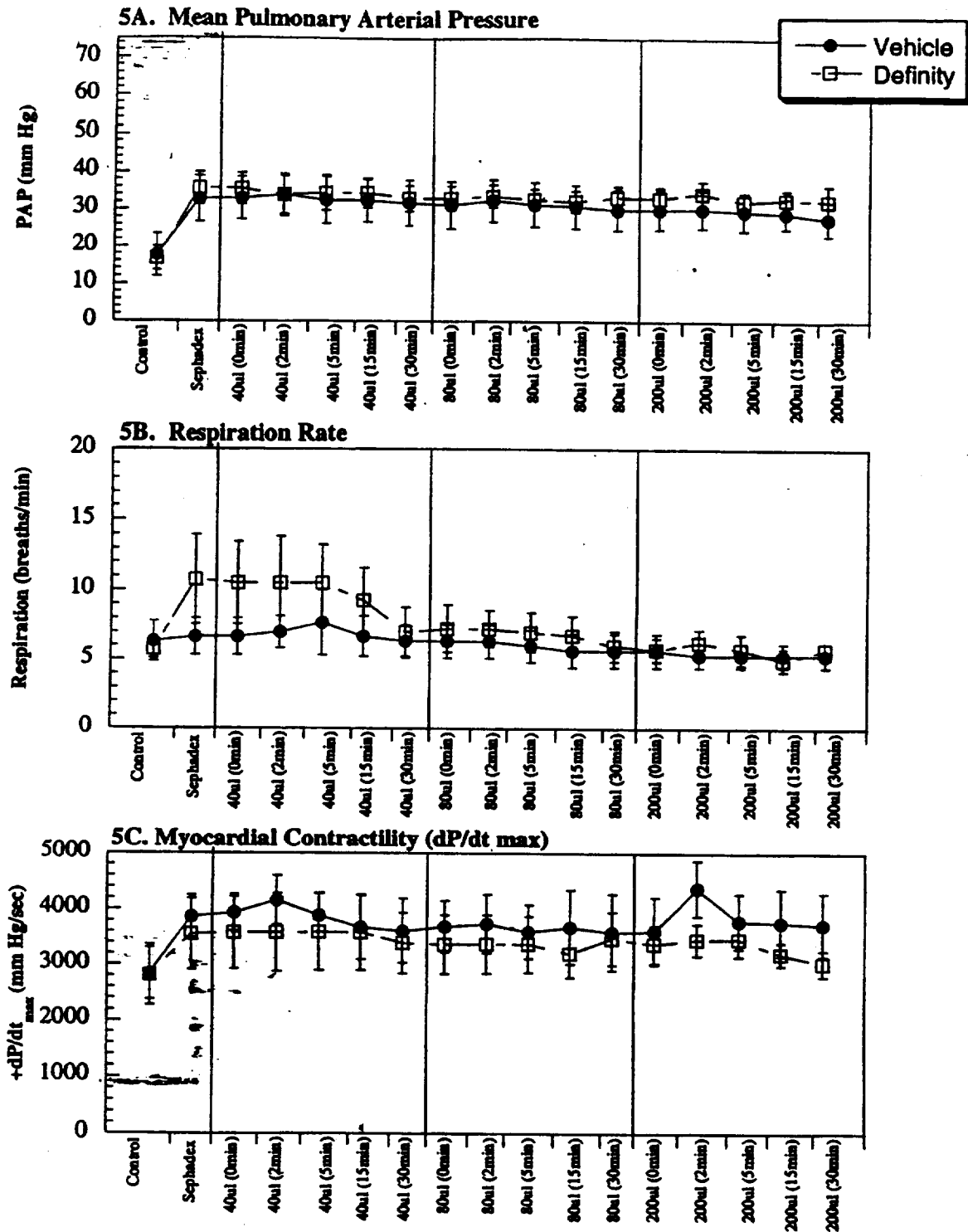


Fig.5. Effects of Definity and vehicle on mean pulmonary arterial pressure, respiration rate and myocardial contractility in spontaneously-breathing anesthetized dogs with moderate pulmonary hypertension(n=3-4)



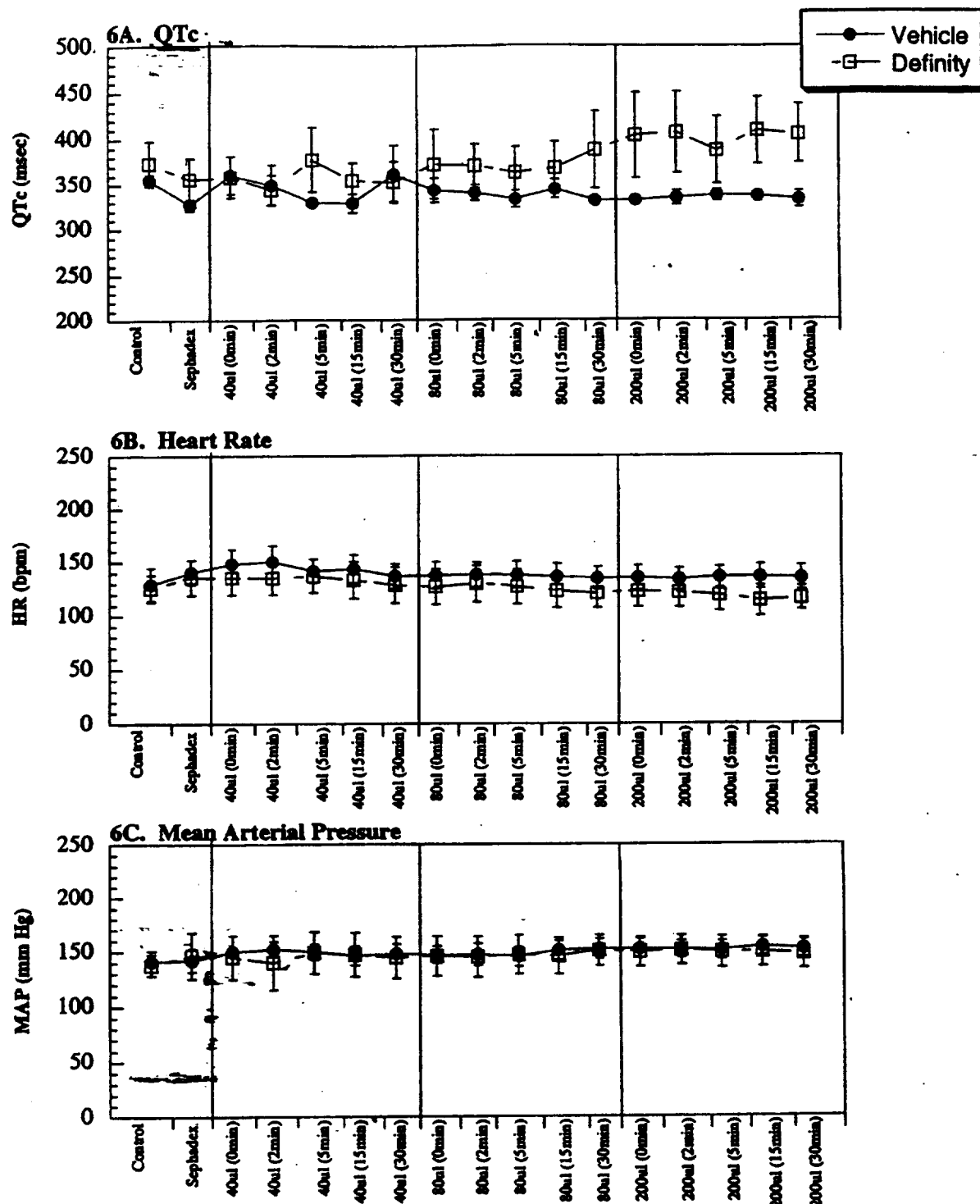


Fig 6. Effect of Definity and vehicle on the corrected QT interval, heart rate, and mean arterial pressure in spontaneously-breathing, anesthetized dogs with moderate pulmonary hypertension (n=3-4)

**The sponsor concluded** that intravenous administration of Definity did not affect pulmonary arterial pressure, MAP, Heart rate or corrected QT interval (Bazett formula) in animals with severe or moderate pulmonary hypertension induced by sephadex.

**Reviewer's comments:** The sponsor provided most of the results of this study in graphical representation only. The individual animal data were not provided. Based on the presented results, I agree with the sponsor that intravenous administration of Definity did not affect pulmonary arterial pressure, MAP or heart rate in animals with severe or moderate pulmonary hypertension induced by sephadex. Definity also appeared not to affect the QT<sub>c</sub> interval in animals with severe pulmonary pressure induced by sephadex (Fig 3a). However, I disagree with the sponsor's conclusion that Definity did not affect the QT<sub>c</sub> interval in animals with moderate hypertension induced by sephadex. A look at Fig 6a would suggest that compared with control animals, there is a prolongation of the QT<sub>c</sub> interval in animals administered Definity. The prolongation seems to be significant specifically after 200 µl Definity where the lower band of the CI does not overlap.

Thus, there is a discrepancy between figure 6a suggesting a prolongation of QT<sub>c</sub> interval by Definity in animals with moderate pulmonary hypertension induced by sephadex, and the sponsor's conclusion that Definity did not affect the QT<sub>c</sub> interval in animals with severe or moderate pulmonary hypertension induced by sephadex. In view of this discrepancy, the agency on 5/7/01 sent a fax to the sponsor, asking for clarification.

Both the agency's letter and the sponsor's response are reproduced in full below.

**FDA Comments to sponsor:**

Definity seems to be affecting the QT<sub>c</sub> interval in animals with moderate pulmonary hypertension. Specifically, after 200µL, Definity appeared to significantly (the lower band of CI does not overlap) affect QT<sub>c</sub> compared with control. However, the results indicated that injection of Definity does not alter QT<sub>c</sub> interval

Please provide an explanation of how this conclusion was reached.

**Sponsor's Response (5/15/01):**

APPEARS THIS WAY  
ON ORIGINAL

In the study, entitled, "A Rising Dose Cardiovascular Assessment Of Intravenously Administered Definity In An Acute Model Of Pulmonary Hypertension In The Anesthetized Closed-Chest Spontaneous Breathing Dog," the data for the corrected QT interval was included. The study report stated that no changes were observed in the QTc interval in either the dogs with moderate or severe pulmonary hypertension. An analysis of variance was performed to determine if the changes observed were due to the administration of DEFINITY™. The analysis of variance was calculated to determine if changes in the QTc, compared to their baseline data, were due to the Sephadex injections or to the administration of DEFINITY™ in those dogs with moderate pulmonary hypertension. We feel this is a more relevant comparison due to inter-animal variability. The p value from the ANOVA for the QTc data in dogs with moderate pulmonary hypertension receiving DEFINITY™ was 0.955, indicating there was no effect of DEFINITY™ administration on the corrected QT interval.

We concur with the reviewer that for the animals with moderate pulmonary hypertension, there appears to be a significant increase in the corrected QT interval when compared to the control animals. However, administration of DEFINITY™ to animals with severe pulmonary hypertension did not result in any change in the corrected QT interval. We, therefore, reviewed the original data for all four animals tested with DEFINITY™ in the moderate pulmonary hypertension arm of the study and carefully examined the lead II electrocardiogram. This examination of the data showed that the data acquisition system had not correctly measured the QT interval for one of the four dogs. The data reflected in the graph, Figure 6A, in the aforementioned report utilized this computer-generated data. We have assessed all intervals by hand measurement and used the Bazett's formula to calculate the corrected QT interval due to heart rate changes. There was no change in the corrected QT interval in the moderate pulmonary hypertension group of dogs treated with DEFINITY™ at any of the doses. An amended graph with the hand measurements is provided.

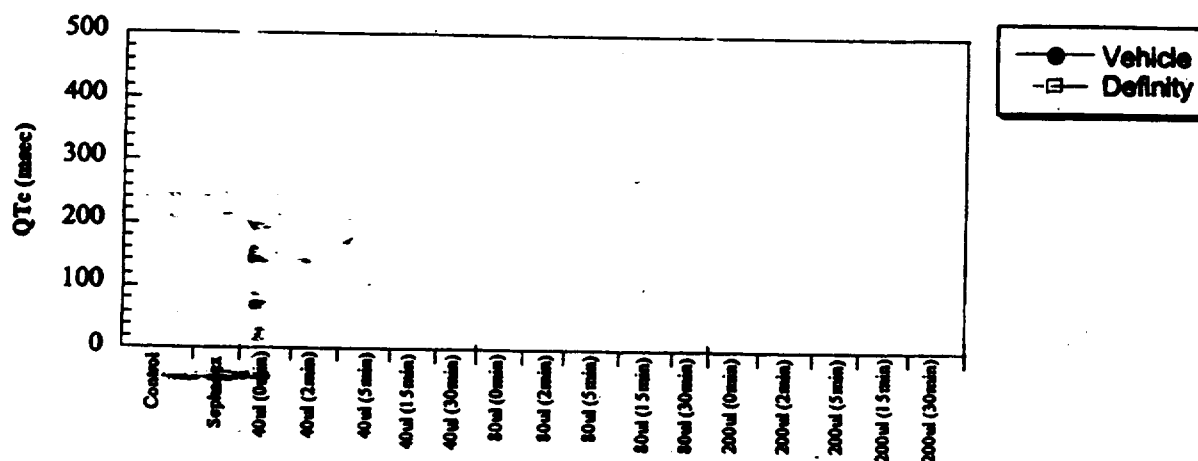


Fig:6A; Effects of Definity ( 40, 80, 200  $\mu$ L/kg IV) on the corrected QT interval. QT interval was measured manually using raw data printouts. Bazett's formula was used to correct interval for changes in heart rate.

**Reviewer's comments on sponsor's response:** The sponsor concurred with the agency that for the animals with moderate pulmonary hypertension, there appears to be a significant increase in the corrected QT interval when compared to control animals. The sponsor also claimed that due to inter animal variability, an analysis of variance to determine if changes in the QT<sub>c</sub> compared to their baseline data, were due to the sephadex injections or to the administration of Definity is a more relevant comparison. The sponsor also asserted an error in measurement for data used to generate the original fig 6a led to the observed difference (please see the sponsor's response).

If the new data are the correct data, I have no alternative other than to agree with the study's conclusion that Definity has no effect on QT<sub>c</sub> interval in animals with moderate or severe pulmonary hypertension caused by sephadex. I also agree with the sponsor that an analysis of variance to determine if changes in the QT<sub>c</sub> compared to their baseline data, were due to the sephadex injections or to the administration of Definity is a more relevant comparison.

From a risk assessment point of view, the worst case scenario would be to believe that the original fig 6 submitted with the NDA was the correct figure. This assumption would imply that there was a statistically significant difference between the control animals, and the animals that received Definity. The large error bars might also suggest that Definity significantly affected the QT<sub>c</sub> interval in at least one of the animals. The question then becomes; what do we do with this information. Given that preclinical data are only signals of what presumably could happen in humans, and the fact that a single pre-clinical animal model is not sufficient to predict the occurrence of drug effect on QT<sub>c</sub> interval in human. The agency can ask for phase 4 commitment from the sponsor to systematically evaluate the effect of Definity on QT<sub>c</sub> prolongation in a pulmonary hypertension model. Additionally, the agency can ask that the present study be audited.

In spite of the equivocal outcome of this study, the study clearly brings into focus the need for heightened awareness of the necessity to monitor the effect of Definity on QT prolongation in humans. Specifically, the study points out the need for contraindicating Definity in patients with pulmonary hypertension.

Finally, it is the considered opinion of this reviewer that further investigation of the effect of Definity on QT prolongation is best studied in humans.

#### **Updates on the QT<sub>c</sub> data:**

During the Definity team internal meeting held on 05/22/01, Dr. Nakissa Sadrieh, supervisory pharmacologist/toxicologist discussed the issue surrounding the QT<sub>c</sub> data. It was decided that a teleconference be held with the sponsor to resolve any outstanding issue with the data.

The teleconference was held on 5/24/01. The agency asked the sponsor about how the error in QT<sub>c</sub> measurement in one dog was discovered and the steps taken to correct the error. The sponsor gave the same explanation as contained in their submission of 5/15/01. The agency then requested that the sponsor submit for evaluation;

- 1) Graphs of the individual animals from the moderate hypertension group
- 2) Tables showing the individual data, both from the manual measurements and the Excel spreadsheets software used for both moderate and severe pulmonary hypertension group.

The requested data were submitted on 5/30/01.

**Reviewer's comments:** Following the review of the submitted data, I agree with the sponsor conclusion that intravenous administration of Definity did not affect pulmonary arterial pressure, MAP, Heart rate or corrected QT interval (Bazzet formula) in animals with severe or moderate pulmonary hypertension induced by sephadex.

**The study is deemed adequate.**

## OVERALL SUMMARY

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed for contrast-enhanced echocardiographic imaging of cardiac structure.

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator. This submission (01/30/2001) by DuPont was in response to the agency's approvable letter of 8/4/2000. The agency advised the sponsor that the application was approvable for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border. The agency identified a number of issues to be addressed by the sponsor before the application is approved. Please note that only the pre-clinical pharmacology aspect of the agency's letter is addressed. For a complete record of the agency's position on this product, please refer to the action letter NDA 21-064 of 04/08/2001.

In general, microbubbles as a class of diagnostic agent are characterized by inherent potential to cause pulmonary embolism with the resultant clinically significant hemodynamic changes. Such hemodynamic changes that may be handled reasonably well in healthy humans may aggravate already compromised functions in special populations such as those with chronically compromised pulmonary functions or pediatrics with immature pulmonary vasculature. The product characteristic of Definity, (largest allowed particles are of 47 µm in diameter) together with the preclinical data submitted for the NDA indicating a low human dose multiple for eliciting hemodynamic and histological effects consistent with pulmonary congestion, led the agency to request from the sponsor the completion of a special pharmacology safety study to evaluate this risk in a chronically compromised pulmonary vasculature model.

The two preclinical studies (DRP 2001-22 and DRR 2001-01) conducted by the sponsor addressed the agency's concern for risk identification in a special population: those with compromised pulmonary function.

The results of the microcirculation study demonstrated that a portion of Definity administered intra arterially become trapped within the microcirculation. The results confirmed the agency's position that studies evaluating microvascular toxicity should be conducted using an intra-arterial mode of administration since after intravenous administration, only microbubbles smaller than the functional diameter of pulmonary capillaries will enter the systemic circulation. The larger particles becoming filtered by the lungs. Thus, clearly indicating that Definity should be contra-indicated in patients with right-to-left, bi-directional, or transient right-to-left cardiac shunt where phospholipid-encapsulated microbubbles can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation. To this end, I recommend that appropriate language be included in the product insert alerting end users to this inherent risk.

The sponsor also examined the effect of intravenous Definity on hemodynamic and myocardial contractility in an animal model of acute pulmonary hypertension. It was concluded that intravenous administration of Definity did not affect pulmonary arterial pressure, MAP, Heart rate or corrected QT interval (Bazett formula) in animals with severe or moderate pulmonary hypertension induced by sephadex. Based on these results, and the results of the high dose safety pharmacology studies conducted in dogs (DRR 20000-01), **the sponsor stated that they have removed the pulmonary emboli language from the warnings section of the package insert (cover letter, Vol.1 page 4).**

The clinical relevance of the sephadex-induced pulmonary hypertension model has not been demonstrated. I therefore, recommend that the pulmonary emboli language be kept in the package insert together with a brief description of the results of the study.

**Overall Conclusions: The sponsor has adequately addressed the pre-approval pre clinical pharmacology/toxicology concerns. Outstanding issues identified below can be addressed with Phase 4 commitments.**

**The application is approvable from pre-clinical pharmacology/toxicology perspectives subject to DuPont's commitment to conduct the following recommended phase 4 studies to determine:**

**The fate of the injected microbubbles:** Human pharmacokinetics information is not available for the intact or degassed lipid microsphere. Preclinical pharmacokinetics studies examined the fate of the octafluoropropane gas or the lipids alone. Thus the fate of the encapsulated liposome in the body is not known. The questions suggested below could be answered with data from either in human or pre-clinical studies:

#### **Pharmacokinetics study:**

How long does the intact microbubble stay in the circulation? The question is of importance in the event of repeat dosing in the same patient since one may not require a full second dose for continued imaging.

#### **Effects on endothelial integrity:**

What are the pathophysiological consequences of ultrasound –microbubbles combination? For example, can energy release from ultrasonographic cavitation process result in cell membrane destruction? Although DuPont examined the potential of Ultrasound energy and Definity combination to cause in vivo hemolysis of red blood cell in dogs, the doses of Definity employed (X 0.3-1 MIPDb/sa) were at the low end of human multiples. Also, the effects on endothelial integrity were not assessed.

#### **Labeling Review:**

Warnings:

**Draft Labeling**

**Reviewer's signature:**

/S/  
Adebayo, A. Laniyonu, Ph.D.  
6/1/01  
Date

**Team Leader Concurrence:**

/S/  
Nakissa Sadrieh, Ph.D.  
6/1/01  
Date

**Appendix/attachments:**

**CC:**

Original NDA  
DIVISION FILES  
HFD-160/LANIYONU/SADRIEH  
HFD-160/ZOLMAN/JONES  
HFD-160/CHO

Concur with Dr. Laniyonu's  
conclusions & recommend  
There are no outstanding  
Pharm Ix issues prior to  
NDA approval.

/S/

6/1/01

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

**KEY WORDS:** Ultrasound contrast agent, Perfluoropropane

**Reviewer Name:** Adebayo Laniyonu, Ph.D.

**Division Name:** Medical Imaging and Radiopharmaceutical Drug Products

**HFD#:** 160

**Review Completion Date:**

**Review number:** #2

**NDA number:** 21-064

**Submission:**

Serial Number	Letter Date	Stamp Date	Type of Submission
000	02/07/2000	02/08/200	AZ
000	03/13/2000	03/14/2000	BZ
000	03/31/2000	04/03/2000	BZ
000	04/21/2000	04/24/2000	BM

**Date:** 2/8/2000

**Type of submission:** N

**Information to sponsor:** Yes ( X ) No ( )

**Sponsor (or agent):**

DuPont Pharmaceuticals Company  
Medical Imaging Division  
331 Treble Cove Road  
North Billerica, MA 01862

**Manufacturer (drug substance):**

**Drug:**

**Code Names:** DMP 115, SG897, MRX-115 and Aerosomes  
**Generic Name:** Perfluoropropane  
**Trade Name:** Definity™  
**Chemical Name:** 1,1,1,2,2,3,3,3-Octafluoropropane  
**CAS Registry Number:** 76-19-7  
**Molecular Weight:** 188.02 g/mol



**Relevant INDs/NDAs/DMFs:**

IND #

DMFs #

**Drug Class:**

Ultrasound Contrast agent

**Indication:**

Echocardiography

**Clinical formulation:**

Vial Component	Concentration/mL
Sodium Chloride	6.8 mg
Propylene Glycol	103.5 mg
Glycerin	126.2 mg
Water for Injection, USP	
*Lipid Blend	0.75 mg
Sodium Hydroxide, NF	As needed to adjust pH
Hydrochloric Acid, NF	As needed to adjust pH
Perfluoropropane Gas	% in the Headspace
Injectate Characteristics	
Perfluoropropane Gas	0.15±0.10 mL
Number of Microbubbles (Clinically useful Range)	$1.2 \times 10^{10}$

**Route of administration:**

Intravenous

**Previous clinical experience:** See medical officer review.

**Disclaimer use of sponsor's material:**

Sponsor submitted texts were utilized in the preparation of this review. They will be identified as quotes

**Studies reviewed with this submission:**

Study T99-2-1: DMP 115: An intravenous range-finding teratology study in the rat. Vol 4 pages 67-213

Study T99-1-7: DMP 115: An intravenous range-finding teratology study in the rabbits. Vol 5 pages 2-163

Study T99-3-4: DMP 115: An intravenous range-finding teratology study in the rabbits. Vol 5 pages 164-503

Study T99-3-3 DMP 115: An intravenous range-finding teratology study in the rat. Vol 6 pages 1-389

Study DRR 2000-01: A rising dose cardiovascular assessment of intravenously administered DMP 115 in anesthetized closed chest dogs. Vol. 7 pages 1-28

Study DRR 2000-02: Microvascular rheology of Definity microbubbles Vol. 7 pages 58-71

Study RDR 98-08: Dose response of DMP 115 and perfluoropropane in conscious rats following an intravenous dose Vol. 7 pages 72-81

Appendix L: Final report from study UOAW-156 entitled, "A rising dose cardiovascular telemetry study of an ultrasound contrast agent intravenously administered to Rhesus monkeys pages 87-158

**Studies not reviewed:**

None

**Introduction and drug history:**

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed for contrast-enhanced echocardiographic imaging of cardiac structure.

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator.

The original NDA was received on 12/09/98. The agency in an action letter dated 10/8/99 advised the sponsor that the application was approvable for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border in doses of 10 µl/kg. The agency identified a number of issues to be addressed by the sponsor before the application is approved. The following non-clinical issues related to safety were identified. Please note that only the pre-clinical pharmacology aspect of the agency's letter is addressed. For a complete record of the agency's position on this product, please refer to the action letter NDA 21-064 of 10/8/1999

**FDA REQUEST:**

**SAFETY**

1. The activated microbubble upper limits of the particle size distribution lack sufficient manufacturing control to ensure safety of the administered product.

The chemistry and animal pharmacology data indicate that the optimal imaging characteristics are dependent predominantly on microbubbles less than 10 µm in diameter. The manufacturing specifications indicate that the largest allowed particles are 47 µm in diameter. Particles of this size are known to be associated with micropulmonary embolism.

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The safety pharmacology section contained several cardiovascular studies that monitored pulmonary and cardiovascular pressures in different species. In all of these studies the human dose multiples were very low. Specifically, the maximum human dose multiples based on body surface area were 0.03 in rats, 0.81 in dogs, 0.5 in pigs, 5.5 in rabbits, and 1.62 in monkeys. Across these species the pulmonary artery pressures were either normal or elevated at these dose multiples. In rats and one dog, there were histologic changes consistent with pulmonary congestion. Because of the low dose multiples of these studies and the inconsistency across species, these studies are not conclusive. While these collective sets of data are not conclusive for the occurrence of micropulmonary emboli, they are suggestive. In order to resolve this deficiency the following are recommended:

Completion of a special pharmacology safety study to evaluate the risk in a chronically compromised pulmonary vasculature disease model. This should include a rigorous assessment at a wide range of human dose multiples adjusted for body surface area. Depending upon the manufacturing approach taken and the results of the special safety study, additional clinical bridging studies may be needed.

#### **SPONSOR'S RESPONSE:**

The sponsor agreed with the agency's position that particles less than  $\geq 10 \mu\text{m}$  may elicit capillary plugging with the attending safety concern for pulmonary embolism.

However, the sponsor contended that there are at least two FDA-approved products that rely on particles to generate diagnostic images with similar particle characteristics as Definity. The sponsor specifically referred to macroaggregated albumin (MAA) and Optison<sup>®</sup> and argued that there is significant clinical experience with these marketed products without reports of extensive pulmonary compromise. Concerning MAA, DuPont stated that approximately 100 million vials of MAA have been sold, and that, no adverse reaction of any kind, including events associated with capillary plugging and micropulmonary embolism have been reported. The sponsor also conducted two studies summarized below. An evaluation of both studies will be provided following the studies summaries.

**A):**

**Study DRR 2000-01:** A rising dose cardiovascular assessment of intravenous administered DMP 115 in pentobarbital-anesthetized closed-chest dogs to determine the effects of high doses of DPM 115 on hemodynamics, myocardial, respiration rates and arterial blood gases.

For this study, dogs of both sexes were prepared for arterial, left ventricular, left ventricular end diastolic, and pulmonary arterial pressure, heart rate, myocardial contractility, respiration and EKG measurements. For DMP 115 administration, the animals were divided into two groups (n=3-4). Group 1 received DMP 115 intravenously at increasing doses of 0.3, 0.5 and 1 ml/kg, (8, 13.5, 27 MHDbsa based on  $20 \mu\text{l/kg}$  dose) at 30 minute intervals. According to the sponsor, the concentration of PFP in the injectate for this group was 193  $\mu\text{l/ml}$ . Group 2 received DMP 115 intravenously at increasing doses of 0.3, 0.5, 1 and 3 ml/kg, at 30 minute intervals. According to the sponsor, the concentration of PFP in the injectate for this group was 53  $\mu\text{l/ml}$ . Apparently, the difference in the concentration of PFP was due to the time interval between product activation and time of injection. The vials for group 1 were allowed to sit for 5 minutes while those for group 2 were allowed to sit for 12 hours. Both groups received unactivated DPM

115 as control. Hemodynamic parameters were monitored continuously while arterial blood gases were determined at end of administration, and at 2, 5, 15 and 30 minutes post-treatment. Group 1 contained a total particle count of 2.4 E9 particles per ml and  $>10\mu\text{m}$  particle count of 1.6E6 particles/ml. Group 2 contained a total particle count of 1.3E9 particles per ml and  $>10\mu\text{m}$  particle count of 0.7 E8 particles per ml. The total number of particles, and the number of particle  $>10\mu\text{m}$  went down by approximately 50% when the vial was allowed to sit for 24 hours. DMP 15 containing 193  $\mu\text{l/ml}$  of PFP at doses 0.3 and 0.5 ml/kg did not produce significant alteration in any of the parameters measured. DMP 115 at 1ml/kg elicited marked changes in the parameters measured as shown in figure 1 and in the following table.

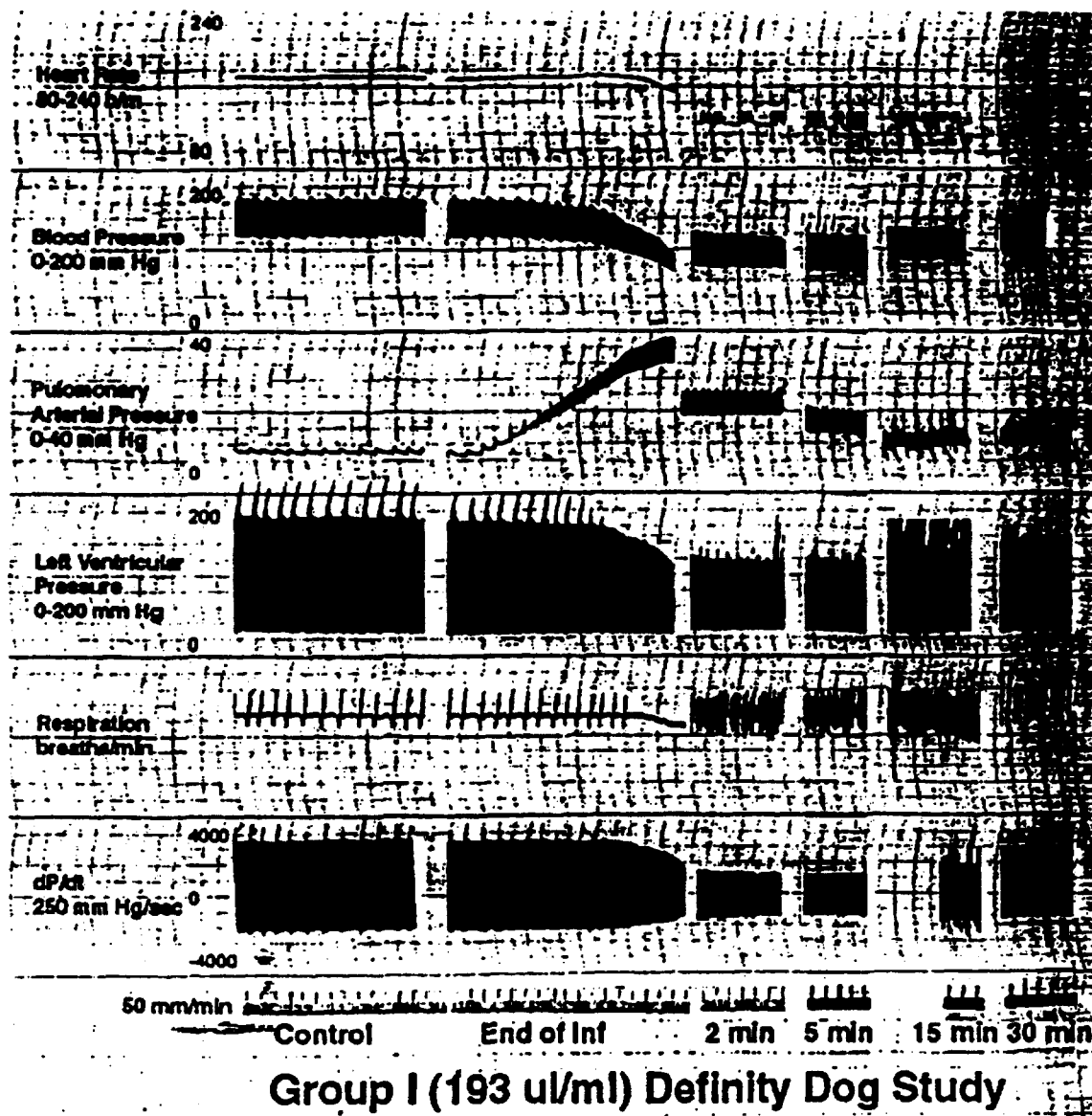


Fig 1: Representative trace of group 1 dogs administered DMP 115, 1ml/kg containing 193  $\mu\text{l/ml}$  PFP. Depicted are the effects on heart rate, arterial pressure, pulmonary pressure, left ventricular pressure, respiration rate and the velocity of contraction.

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Parameter	Pre-DMP 115	Post-DMP115	% Change
Respiration rate	12±5	48±15	300
Pulmonary systolic Arterial pressure	8±0.3	23±10	188
Mean arterial pressure	122±11	68±16	44
Left ventricular pressure	164±7	109±20	34
Myocardial contractility	2913±542	1450±340	50

All parameters returned to their respective control level by 30 minutes post-treatment. According to the sponsor, there were no significant changes in arterial blood gases and the changes in mean arterial pressure and myocardial contractility were secondary to the increases in pulmonary arterial pressure. One of the four dogs treated with 1 ml/kg died 15 minutes post-treatment. NOEL was 0.5 ml/kg (MHD 13.5 based on body surface area). When corrected for the maximum allowable quantity of PFP in the product (baseline maximum specification 250µl/ml )MHD is 10.4.

For the group 2 animals there were no significant changes from controls in all parameters that were measured.

**Table 1. Summary PFP content and particle size distribution for DMP115 lot 4514MZ**

	Group I	Group II
PFP (µl/ml)	193 ± 7	51 ± 4
Particle Size [ ] µm/ml		
1 < 2	2.2 ± 0.3 E9	1.2 ± 0.6 E9
2 < 6	1.3 ± 0.1 E8	1.1 ± 0.4 E8
6 < 10	2.2 ± 0.6 E7	1.6 ± 0.9 E7
≥ 10	1.6 ± 1 E6	0.7 ± 0.5 E6
Total	2.4 ± 0.3 E9	1.3 ± 0.7 E9

a. All values are expressed as the mean ± SEM. Data were derived from stability program using the 6-9 month timepoints. PFP determination was performed using protocol SOP17212015. Particle sizing was performed using SOP17212021.

The sponsor also ruled out the effect of particle size since more microbubbles were administered for group 2; 3ml/kg compared with group 1; 1ml/kg.

The sponsor ruled out an acute anaphylactoid response since unactivated vial did not alter any of the parameters examined.

The sponsor concluded that the results demonstrated that the group 1 DMP 115 sample containing 193  $\mu$ l PFP at a dose of 1ml/kg elicited marked changes in respiration rate and pulmonary arterial pressure of 300% and 188% respectively. These increases were accompanied by secondary decrease in myocardial contractility and mean arterial pressure. DMP115 containing 51 $\mu$ l PFP/ml at 3ml/kg did not significantly alter any of the parameters studied. Therefore, the changes observed were due to the higher concentration of PFP gas contained in DMP 115 that was administered to group 1 animals.

**B):**

**Study: DRR2000-02**

**The sponsor also submitted a study entitled: Dose-response of DMP 115 and perfluoropropane in conscious rats following intravenous dose.** This study was reviewed with the original submission and a summary is provided here. The sponsor investigated the role played by PFP gas in the acute toxicity of the product. The underlying hypotheses were 1) The PFP content of the administered DMP 115 formulation might in part be responsible for the clinical signs and death attributed to DMP 115 in rats. 2) The longer the time interval between activation and injection, the higher the probability of allowing more of the PFP gas to diffuse out of activated DMP 115 formulation resulting in lowered toxicity (explaining the role that time interval between activation and injection plays in the elicitation of toxicity). PFP-containing headspace was injected intravenously. There were several deaths. Clinical signs observed prior to death included rapid breathing, dyspnea, ataxia, decreased motor activity, and loss of writhing reflex. Symptoms that were remarkably similar to those elicited in rats by DMP 115. There was poor correlation between the individual PFP concentrations, and time between activation and injection. The sponsor used this lack of correlation, and the fact that direct intravenous injection of PFP-containing head space was not the optimal model to evaluate the potential toxicity of PFP gas in DMP 115 injectate to conclude that the results regarding the role of PFP in toxicity is equivocal.

**Reviewer's comments to both studies submitted in response to deficiency:**

**Safety Implication:**

In general, microbubbles as a class of diagnostic agent are characterized by inherent potential to cause pulmonary embolism with the resultant clinically significant hemodynamic changes. Such hemodynamic changes that may be handled reasonably well in healthy humans may aggravate already compromised functions in special populations such as those with chronically compromised pulmonary functions or pediatrics with immature pulmonary vasculature. The product characteristic of Definity, (largest allowed particles are of 47  $\mu$ m in diameter) together with the preclinical data submitted for the NDA indicating a low human dose multiple for eliciting hemodynamic and histological effects consistent with pulmonary congestion led the agency to request from the sponsor the completion of a special pharmacology safety study to evaluate this risk in a chronically compromised pulmonary vasculature model.

The two preclinical studies conducted by the sponsor failed to address the agency's concern for risk identification in a special population: those with compromised pulmonary function.

For the dog study, the sponsor concluded that the increased respiration rate and pulmonary arterial pressure at high doses of DMP 115 is attributable solely to the PFP gas administered. In the study evaluating the role of PFP gas in DMP 115 toxicity in rats, injection of PFP-containing headspace resulted in death of several rats. Clinical signs observed prior to death included rapid breathing, dyspnea, ataxia, decreased motor activity, and loss of writhing reflex. Symptoms that were remarkably similar to those elicited in rats by DMP 115.

Overall, the sponsor stated inter alia that "DMP115 safety margin, from this study based on the maximum quantity of PFP gas allowed by the specifications of the product (250 µl/ml) is 19.3 based on body weight and 10.4 based on body surface area. Therefore, the product does not pose a risk to man when administered at the intended clinical dose".

**The studies main conclusion that the adverse pulmonary and cardiovascular events are attributable to the PFP component of the formulation has not answered the primary question posed by the agency concerning the safety of this product in animal models with compromised pulmonary functions.**

To begin with, the preclinical Pharmacology/Toxicology studies that led the agency to state in its letter of 10/8/99 that

*"The safety pharmacology section contained several cardiovascular studies that monitored pulmonary and cardiovascular pressures in different species. In all of these studies the human dose multiples were very low. Specifically, the maximum human dose multiples based on body surface area were 0.03 in rats, 0.81 in dogs, 0.5 in pigs, 5.5 in rabbits, and 1.62 in monkeys. Across these species the pulmonary artery pressures were either normal or elevated at these dose multiples. In rats and one dog, there were histologic changes consistent with pulmonary congestion. Because of the low dose multiples of these studies and the inconsistency across species, these studies are not conclusive. While these collective sets of data are not conclusive for the occurrence of micropulmonary emboli, they are suggestive"*

were conducted at appropriate concentration of PFP in the formulation. Moreover although the sponsor asserted that the concentration of PFP that led to manifestation of adverse pulmonary and other cardiovascular changes was far in excess of that encountered in normal clinical setting. How an animal model with compromised pulmonary function would have reacted was not studied. It seems reasonable to conclude that elicitation of adverse pulmonary events in such animal models might occur at lower DMP concentrations containing high amounts of PFP gas.

The sponsor also referenced both Optison and macroaggregated albumin as a basis for disagreeing with the agency's conclusion that particles with diameters > 10µm may result in clinically meaningful insult to the pulmonary system. In my opinion, these two products irrespective of the fact that they are already on the market should not be used as a basis for not assessing the safety profile of a new to be marketed formulation in a special population. Macroaggregated albumin is indicated as an adjunct in the evaluation of pulmonary perfusion and is contraindicated in patients with severe pulmonary hypertension. Moreover the agency is in possession of proprietary data that raised concerns about the potential safety risk of the microbubble contrast agents due to microbubble size, concentration or volume amongst other factors. Therefore with time, as we gather more information on microbubbles as a class, our requirements for safety data are subject to change, based on this proprietary information.

**I therefore conclude that there is still a need for asking the sponsor to conduct a study in animal models with compromised pulmonary function.**

**Labeling Implication:**

The sponsor's response failed to address the agency's concern regarding risk identification in a special population: those with compromised pulmonary function. However, it succeeded in highlighting another concern first raised in my overall summary of the NDA; how the condition of clinical use for this product was determined especially as regards the time interval between product activation and administration.

Taken together, the two studies confirmed the critical importance of PFP gas in the manifestation of the overt signs of toxicity elicited by the animals in the various toxicological studies submitted for the NDA. More importantly, it again supported my opinion as stated on page 90 of my primary review that the time interval between product activation and injection may contribute to the manifestation of DMP 115 toxicity. It bears emphasizing that the difference in the concentration of PFP between the high PFP group and the low PFP group in the dog study resulted from the time interval between product activation and time of injection. The vials for group 1 were allowed to sit for 5 minutes while those for group 2 were allowed to sit for 12 hours.

From the review of the original NDA, it is reasonable to conclude that efficacy was not compromised by the time interval between product activation and administration. In preclinical animal studies, DMP-115 remains efficacious in producing echocardiographic demonstration of left ventricles and of myocardial perfusion following activation through mixing up to four days post-preparation. The responses produced at later stages following preparation are comparable to the response obtained immediately post preparation despite the fact that the number of particles per ml remaining was less, suggestive that a lower concentration of DMP 115 might be sufficient to obtain clinical efficacy (page 83 of my original review).

Injecting DMP 115 five minutes after product activation led to the injection of DMP 115 containing a high amount of PFP gas with the resultant increase in pulmonary insult. The pulmonary insult is absent when the activated product is allowed to sit for a longer time period.

To this end, I am of the opinion that the time interval between activation and injection is a labeling issue. The agency should request that the sponsor stipulate a minimum time interval between product activation and administration.

**In conclusion, the studies submitted by the sponsor to address this deficiency are inadequate to assess the safety of this product in animal models with compromised pulmonary function. Therefore there is still a need to request that the sponsor conduct a study addressing the safety of Definity in animal models with compromised pulmonary function. Moreover, based on the results of the studies submitted, the agency should request that the sponsor stipulate a minimum time interval between product activation and administration.**



**FDA request:**

**2. The application lacks sufficient data to characterize the risk of arrhythmias.**

The animal safety pharmacology studies used to evaluate electrocardiographic and contractility parameters were the same studies discussed in above. Therefore, the dose multiples are too small to support definitive conclusions. Also, there were two toxicology studies that used higher doses (48 MHD and 162 MHD). In these studies there were findings of ventricular tachycardia, AV block, and bundle branch blocks. However, these studies were not designed to comprehensively evaluate the cardiovascular system. As such, the pre-clinical database does not contain sufficient information upon which to base the risk of acute cardiovascular adverse events. Also, data on the potential for systemic clumping, aggregation or coalescence was not provided. Therefore, the evaluation of the risk of QTc prolongation, rhythm disturbances or other myocardial conduction abnormalities can not be completed. In order to resolve these deficiencies the following are requested:

The completion of a safety pharmacology cardiovascular study using a wide range of human dose multiples based upon body surface area. This may be accomplished in conjunction with the pulmonary vasculature study requested in the preceding section.

An *in vivo* evaluation of the potential for clumping, aggregation or coalescence in the systemic circulation (e.g., a microvascular model such as a mesenteric artery, cheek pouch, or retinal vessels).

**SPONSOR'S RESPONSE:**

In response to this deficiency, the sponsor conducted a cardiovascular study titled;

**A): Study UOAW-156:**

**"A rising dose cardiovascular telemetry study of an ultrasound contrast agent administered intravenously to rhesus monkeys"**

Male monkeys (n = 4) were implanted with telemetry capable of monitoring various cardiovascular parameters including blood pressure, heart rate and EKG. Pulmonary vasculature was not examined. Animals were infused cumulatively with Definity at 0.3, 0.5 and 1.0 ml/kg (4.8-16 MHDbsa) at a rate of 2 ml/min. In the original study submitted for the NDA, the rate of infusion was 3 ml/kg.

There was no significant effect on MAP, heart rate or EKG. Based on these results the sponsor concluded that the NOEL for the study was 1.0 ml/kg (x16 MHDbsa).

**B): Study #DRR 98-08**

The sponsor addressed the issue of the potential for DMP 115 to cause clumping, aggregation or coalescence in the systemic circulation in a study conducted by Jonathan, R. Lindner, MD and his co-workers at the University of Virginia school of medicine and titled: **Microvascular rheology of Definity microbubbles:**

Mice cremaster muscle was prepared for intravital microscopic study. The muscle was exteriorized through a scrotal incision and secured to a pedestal. Epifluorescent imaging was performed using a 530-560 nm excitation filter. Microbubble velocity was measured off-line using a custom designed computer program. Definity microbubbles were labeled with a yellow fluorescent tag (PKH26) with a mean excitation wavelength of 551 nm. Approximately 20 $\mu$ l of microbubbles was injected intravenously over 15 sec. The median diameter for arterioles and venules in which RBC and microbubble velocity were measured was 22 $\mu$ m. The study showed a close correlation between mean centerline RBC velocity and mean microbubble velocity for both arterioles and venules. Microvascular obstruction by microbubbles was not observed in any of the fields of observation. According to the authors, transit of Definity through the capillary system appeared to be exclusively in the form of single bubbles. There was no observation of microbubble aggregation or coalescence. The video recording of the microvascular study requested by me from DuPont was not helpful as the recording only showed a clip of the experiment and not a complete experiment from the beginning to the end of study.

The sponsors concluded that Definity microbubbles transit through normal capillaries as a single microbubble, and do not cause any capillary or other microvascular obstruction after intravenous administration and pulmonary passage.

#### **Reviewer's comments:**

For the monkey cardiovascular study, I agree with the sponsor's conclusion that there was no significant effect on MAP, heart rate or EKG. The highest dose employed for the study, 1.0 ml/kg did not cause any adverse cardiovascular response. Based on these results, the sponsor concluded that the NOEL for the study was 1.0 ml/kg (x16 MHDbsa). It would have been helpful to use a dose of Definity that would have elicited adverse cardiovascular events, however, it is pertinent to consider electrocardiograph results (T98-7-1) submitted as part of the original NDA. T98-7-1 results were obtained as part of toxicological studies in monkeys conducted using the final to be marketed formulation. All animals (n=6) intravenously administered DMP115 at 3 ml/kg (X48.6MHDbsa) showed abnormal electrocardiograms. Each monkey demonstrated evidence of ST-T segment depression within one minute of the start of infusion followed by the development of ventricular extrasystoles, ventricular tachycardia, first degree and complete atrioventricular block and transient development of right bundle branch block. The ST-T depression began to return toward baseline between 8 -10 minutes of the beginning of drug infusion but persisted longest in one female monkey. All animals received supplemental oxygen for 3 to 6 minutes. No NOEL was determined for the study. The present result, taken together with T98-7-1 can be used to define NOEL at 1 ml/kg ( X16 MHDbsa).

**The study is deemed adequate.**

The mouse cremaster muscle study evaluating the microvascular rheology of Definity microbubbles concluded that Definity microbubbles transit through normal capillaries as a single microbubble, and do not cause any capillary or other microvascular obstruction after intravenous administration and pulmonary passage. It is noted that Definity was administered intravenously; the study should have been conducted with the injection given intra-arterially. Such an approach would have been helpful for risk identification especially in cases of patients

with right-to-left, bidirectional, or transient right-to left cardiac shunt where phospholipid-encapsulated microbubbles can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation. Moreover, the total number of microbubbles or the particle size distribution characteristic of Definity used for the study was not provided. The median diameter for arterioles and venules in which RBC and microbubble velocity were measured was 22  $\mu\text{m}$ . These are rather large vessels to use to measure the potential for Definity to cause clumping, aggregation or coalescence in the systemic circulation. Ideally, the vessel size should be less than 10  $\mu\text{m}$ .

**I therefore conclude that this study is inadequate. The sponsor should be requested to repeat the study.**

**FDA request:**

- 3. The submission does not include data on reproductive toxicology studies performed with the final to-be-marketed formulation. We note that you plan to submit such data to FDA in the first quarter of year 2000.**

**SPONSOR'S RESPONSE:**

The sponsor conducted segment II range-finding and definitive developmental studies in rats and rabbits.

Study T99-2-1: DMP 115: An intravenous range-finding teratology study in the rat. Vol 4 pages 67-213

Study T99-1-7: DMP 115: An intravenous range-finding teratology study in the rabbits. Vol 5 pages 2-163

Study T99-3-4: DMP 115 An intravenous teratology study in the rabbits. Vol 5 pages 164-503

Study T99-3-3 DMP 115: An intravenous teratology study in the rat. Vol 6 pages 1-389

For the rat study, 3.0 ml/kg (x24MHD bsa) resulted in maternal death. At doses  $\leq 1.0$  ml/kg/day (from day 6-17 of gestation), there was no evidence of DMP 115-related maternal or developmental toxicity (fetal growth, survival and morphological development).

For the rabbit definitive study, 1.0 ml/kg/day (x15MHD bsa) resulted in maternal death which was not observed in the range-finding study. Transient maternal toxicity (labored respiration and increased respiration rate) were observed at 0.3 ml/kg (x4.5 MHD bsa). There was no evidence of DMP-related developmental toxicity (fetal growth, survival, and morphological development at 0.3 ml/kg (x4.5 MHD bsa). The fetuses from animals that survived 1.0 ml/kg/day did not show evidence of DMP-related developmental toxicity.

Species	Maternal NOAEL	Fetal NOAEL
Rat	1 ml/kg (x24 MHDbsa)	1 ml/kg (x24 MHDbsa)
Rabbit	Not established in definitive study, 1 ml/kg (x15 MHDbsa) in the range-finding study	1 ml/kg (x15 MHDbsa)

The sponsor did not conduct new studies to evaluate potential effects on fertility and reproductive performance. The sponsor reasoned that:

- Effect on fertility is not an issue for an agent intended for acute diagnostic use.
- DMP 115 did not cause any adverse effects on fertility and reproductive performance in segment I study in rats conducted with the old formulation.
- DMP 115 did not cause any adverse effects in gonads or other reproductive tissues in 1-month toxicity studies in rats and monkeys (T97-11-5, T98-3-46, T98-5-2)

Based on these results, DuPont is requesting for a Pregnancy Category B language to be added to the Definity labeling.

**Reviewer's comments:**

I agree with the study conclusions. The out come of the reproductive toxicology studies result conducted with the final to be marketed formulation are similar to those first submitted with the NDA using an earlier formulation. For both sets of studies, none of the findings were suggestive of adverse effects on reproductive and developmental capabilities.

**I recommend approval of DuPont's request for Pregnancy category B language.**

**OVERALL SUMMARY**

Definity™ (DMP-115) is a lipid-encapsulated perfluoropropane (PFP) microbubble suspension proposed for contrast-enhanced echocardiographic imaging of cardiac structure

The drug product is composed of 2 final intermediates, a lipid blend and perfluoropropane gas suspended in a matrix of sodium chloride, propylene glycol and glycerol in water for injection. It is prepared by shaking with a modified dental amalgamator. The original NDA was received on 12/09/98. The agency in an action letter dated 10/8/99 advised the sponsor that the application was approvable for use in patients with suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border in doses of 10 µl/kg.

In general, microbubbles as a class of diagnostic agent are characterized by inherent potential to cause pulmonary embolism with the resultant clinically significant hemodynamic changes. Such hemodynamic changes that may be handled reasonably well in healthy humans may aggravate already compromised functions in special populations such as those with chronically compromised pulmonary functions or pediatrics with immature pulmonary vasculature. The product characteristic of Definity, (largest allowed particles are of 47  $\mu\text{m}$  in diameter) together with the preclinical data submitted for the NDA indicating a low human dose multiple for eliciting hemodynamic and histological effects consistent with pulmonary congestion, led the agency to request from the sponsor the completion of a special pharmacology safety study to evaluate this risk in a chronically compromised pulmonary vasculature model.

The two preclinical studies (DRR 2000-01 and DRR 2000-02) conducted by the sponsor failed to address the agency's concern for risk identification in a special population: those with compromised pulmonary function. The studies main conclusion that the adverse pulmonary and cardiovascular events are attributable to the PFP component of the formulation has not answered the primary question posed by the agency concerning the safety of this product in animal models with compromised pulmonary functions. To this end, I am recommending that the sponsor be asked to conduct a special pharmacology safety study evaluating the risk for the occurrence of micropulmonary emboli in a chronically compromised pulmonary vasculature disease model.

The mouse cremaster muscle study (DRR 98-08)) evaluating the microvascular rheology of Definity microbubbles concluded that the rheologic properties of intravenously administered Definity is similar to that of Red blood cells. That Definity transit through the capillary bed unimpaired and as single microbubbles. While I agree with the sponsor's general conclusion about the outcome of this experiment as conducted, it is noted that Definity was administered intravenously. The study should have been conducted with the injection given intra-arterially. Such an approach would have been helpful for risk identification especially in cases of patients with right-to-left, bidirectional, or transient right-to left cardiac shunt where phospholipid-encapsulated microbubbles can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation. To this end, I am recommending that the sponsor be asked to repeat the study using intra-arterial route of administration.

This submission highlighted a concern first raised during the review of the original NDA: how the condition of clinical use for this product was determined especially as regards the time interval between product activation and administration.

Both the dog cardiovascular study and the dose response of DMP 115 and perfluoropropane in conscious rats (DRR 2000-01 and DRR 2000-02) confirmed the importance of PFP gas in the manifestation of the overt signs of toxicity elicited by the animals. The studies supported my opinion as stated on page 90 of my primary review that the time interval between product activation and injection may contribute to the manifestation of DMP 115 toxicity. It bears emphasizing that the difference in the concentration of PFP between the high PFP group and the low PFP group in the dog study resulted from the time interval between product activation and time of injection. The vials for group 1 were allowed to sit for 5 minutes while those for group 2 was allowed 12 hours. Consequently, group1 animals were administered a higher concentration of PFP gas, and as a result showed significant hemodynamic alterations as compared to group 2 dogs.